Harnessing Methanotroph-Photoautotroph Interactions for Biogas Conversion

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Project Goals: In nature, microbial communities have developed a highly efficient way to recover energy and capture carbon from both CH_4 and CO_2 through interspecies coupling of methane oxidation to oxygenic photosynthesis. However, in order to successfully utilize mixed culture for biotechnology applications, both fundamental knowledge and technological gaps have to be addressed. The knowledge gap refers to the lack of systematic study for identifying and quantifying the interactions between community members and how the interactions affect system dynamics. The technological gap refers to the lack of effective methodology, and fast and low-cost analytical tools to characterize mixed culture systems frequently or in real-time. The overall objective of this research is to help address those gaps through developing experimental and computational tools to characterize a synthetic methanotroph-photoautotroph (M-P) binary consortium, to identify and validate interspecies interactions at both systems and cellular levels, and to engineer a model methanotroph-photoautotroph coculture pair for enhanced production of chemicals.

Abstract: Biogas derived from organic waste streams through anaerobic digestion has immense potential as a renewable feedstock for producing high-density liquid fuels and commodity chemicals. However, effective and economical biogas utilization has been challenging due to the presence of contaminants. In this project, we have clearly demonstrated that M-P cocultures offer a flexible platform for highly efficient biological CH_4-CO_2 co-utilization and enable significantly enhanced cell growth of both species in a model M-P coculture[2-5]. Beyond the designed interspecies exchange of in situ produced O_2 and CO_2, we hypothesize that there exist other emergent metabolic exchanges that enabled the observed enhanced coculture growth. Through designed experiments and a semi-structured kinetic modeling approach, we successfully validated our hypothesis and quantified the effect of emergent interactions within the model coculture on cell growth, without knowing what the emergent metabolites exchanges are.

Genome-Scale Metabolic Modeling of the methanotroph-photoautotroph coculture

Once the existence of the unknown emergent interactions is confirmed, the next question is how to postulate/identify the potential metabolites that are exchanged within the M-P coculture. It is very challenging to answer this question via experimental approach alone, as the key metabolite being exchanged may not be detectable – the metabolites being produced by one species could be completely and immediately consumed by the other. To address this challenge, we explore a fully structured modeling approach, i.e., genome-scale metabolic modeling for the coculture.

Genome-scale metabolic models (GEMs) represent extensive knowledge bases of microorganisms and provide a platform for model simulations and integrative analysis of different sources of data, including various omics data. In addition, it offers a convenient and powerful tool to generate and test various hypotheses regarding "metabolic links" within the M-P coculture. Using *Methylomicrobium buryatense* - *Arthrosipira platensis* as the model coculture, we have developed the very first GEM model for the M-P coculture using SteadyCom. The coculture GEM was validated by comparing the model predicted individual growth rates with experimental measurement, while using the cross-membrane fluxes for major substrates uptake as constraints.

The coculture GEM is able to predict the change in species abundance and consequently the population ratio of the coculture in response to changes in defined nutrient and carbon substrate to the model. In addition, the coculture GEM is able to predict the "metabolic links" within the coculture. For the model M-P coculture, our GEM predicted 19 metabolites being exchanged between the methanotroph and photoautotroph. The predicted metabolic exchanges include organic acids, amino acids, as well as key metabolite involved in central carbon metabolism. These model predicted metabolic links offer the specific targets to be tested via designed experiments. Currently, we are conducting experiments to collect meta-transcriptomic profiles of the coculture to validate these model predicted exchanges.

A novel circulating coculture biofilm photobioreactor(CCBP): addressing the engineering challenges associated with the M-P coculture-based biotechnology for biogas conversion

Despite the many advantages offered by the M-P coculture for biogas conversion, several long standing technical and engineering challenges have to be addressed before the coculture-based biotechnology can be successfully commercialized. Specifically, mass transfer resistance associated with gas phase substrate and light attenuation in liquid severely limit the achievable cell density and scale up of the coculture-based biotechnology. In addition, the high energy cost associated with biomass harvesting is another major challenge.

To address these long standing challenges, we proposed to explore biofilm-based cultivation, and have developed a patent pending circulating coculture biofilm photobioreactor (CCBP) (USPTO patent application # 16,934,766, filed on July 21, 2020 [1]). The CCBP is designed to effectively address the challenges associate with gas substrate, light attenuation, and biomass harvesting, by offering the following major advantages. (1) By exposing the coculture biofilm directly to the gas phase, the mass transfer resistance from the gas phase to the cells can be significantly reduced, which eliminates the energy intensive agitation or aeration process. (2) The vertically arranged biofilm not only provides high biomass production area with low land footprint requirement, which allows effective scale up without light availability limitation, but also enables sun light dilution and reduce light inhibition. (3) Biomass harvesting can be achieved easily through a retractable scraping blade, therefore significantly reduce the energy and cost required for biomass harvesting. (4) Extracellular polymeric substance (EPS) and emergent properties of the biofilm could enhance the tolerance of the M-P coculture to culture stress and inhibitors.

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